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Modified Procedure for Determining the Forms of Phosphorus in Freshwater Sediments

T. Mayer and J. D. H. Williams*

* This work was initiated and developed by the late Dr. J.D.H. Williams, who died tragically on February 23, 1979.

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Figure 1. Analytical procedures used to determine apatite phosphorus, nonapatite inorganic phosphorus (NAI-P), organic phosphorus (organic-P) and total phosphorus (total-P) 1

Abstract

An analytical procedure for the determination of various forms of phosphorus in sediments is described. The method has been widely used on a variety of samples such as lacustrine and fluvial sediments, bluff material and some pure phosphorus minerals. It is suitable for the analysis of a large number of samples, because it is possible to carry out extraction in batches and the determinations on the extracts can easily be automated.

Résumé

Le présent rapport décrit un processus analytique pour la détermination des diverses formes du phosphore présentes dans les sédiments. La méthode a été largement utilisée sur une variété d'échantillons tels que les sédiments fluviatiles et lacustres, les matières de berge et quelques minéraux de phosphore à l'état pur. Elle convient à l'analyse d'un grand nombre d'échantillons, car il est possible de procéder à l'extraction en vrac et il serait facile de déterminer les extraits par automation.

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INTRODUCTION

An analytical procedure for the determination of the forms of phosphorus in sediments was developed at Canada Centre for Inland Waters, Burlington, by modification of the procedure described in Chang and Jackson (1957) and Williams and Mayer (1972). The authors used this method widely in their most recent work on freshwater sediments, bluff materials, and pure phosphate minerals that are common in soils and sediments.

Figure 1 outlines the analytical procedures employed to determine "apatite phosphorus" (apatite-P), "non-apatite inorganic phosphorus" (NAI-P), and organic phosphorus (organic-P) as well as "total phosphorus" (total-P).

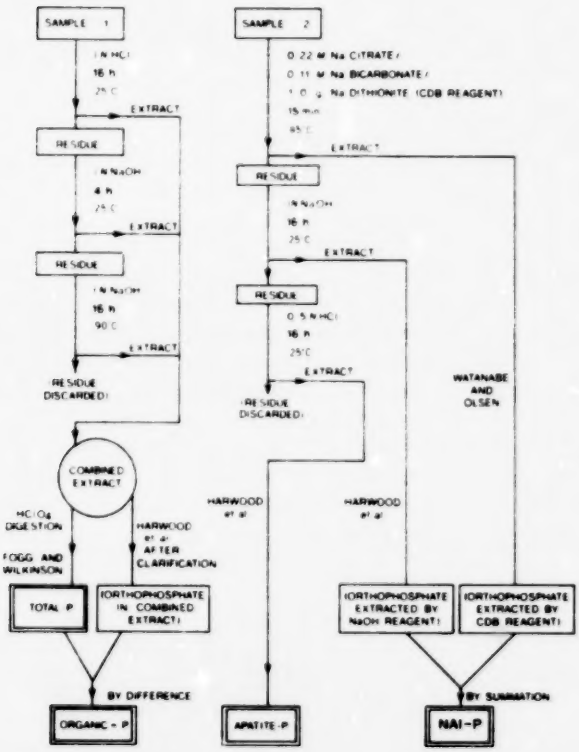

Figure 1. Analytical procedures used to determine apatite phosphorus, nonapatite inorganic phosphorus (NAI-P), organic phosphorus (organic-P) and total phosphorus (total-P).

In this report, the term "apatite phosphorus" denotes the orthophosphate that is present in crystal lattices of apatite grains. Apatite minerals with a low degree of crystallinity tend to be determined as part of the NAI-P fraction (Williams *et al.*, 1980). The expression "total phosphorus" denotes total extracted phosphorus. This value is slightly less than the true total phosphorus content of sediments. The difference is due to forms of phosphorus not extracted during the organic-P procedure (Williams *et al.*, 1976).

Two separate procedures [(1) and (2)] are used to determine orthophosphate in the samples, which represents the major proportion of inorganic phosphorus. The agreement between the results obtained by both methods is generally very good.

The NAI-P + apatite-P approximately equals orthophosphate in combined extract (within 5%-10%). It is time efficient to handle samples in batches of 24. In most cases, after each extraction step, the phosphorus content of the extract was determined by methods which are described later. Automation of some determinations (Harwood *et al.*, 1969; Appendix A) increases the efficiency of the procedure.

Analyses were performed on the freeze-dried samples of 100-mesh particle size. Not much variation of phosphorus content was found with increasing particle size (30 mesh) as long as the sample was sufficiently homogeneous. Interferences were caused by high amounts of arsenic in the samples, which led to the overestimation of phosphorus content. In the majority of treated materials, arsenic content was not high enough to affect the results of phosphorus determinations seriously.

APPARATUS

Nalgene (polypropylene) centrifuge tubes (100 mL) with a stand.
Rubber stoppers (6½) to fit the centrifuge tubes.

Shaker for 24 100-mL centrifuge tubes (end-over-end or wrist action shaker).
Water bath.
Centrifuge (I.E.C. model PR-6).
Volumetric flasks (25 mL, 50 mL, 100–250 mL).
Separatory funnels (125 mL).
Erlenmeyer flasks (125 mL).
Small glass funnels.
UV-visible spectrophotometer absorption cell with 10-mm path length (Pye Unicam SP6-300).
Graduated cylinders or dispensers.
Pipettes.
Glass stirring rods.
Spatulas.
Weighing boats.
Analytical balance (to about 200 g) accurate to 0.5 mg.
Oven with temperature control.
Water pump.
Vortex mixer.
Hot plate.

REAGENTS REQUIRED FOR FRACTIONATION PROCEDURE

Conc. HCl.
Conc. H_2SO_4.
Conc. $HClO_4$ (72%).
1 *N* HCl solution: Dilute 83 mL of conc. HCl to 1000 mL with distilled H_2O (for batch of 24 make up 2.5 L).
3 *N* HCl solution: Dilute 249 mL of conc. HCl to 1000 mL with distilled H_2O.
1 *N* NaOH solution: Dissolve 40 g of NaOH in 1000 mL of H_2O (for batch of 24 make up 4 L).
5 *N* NaOH solution: Dissolve 200 g of NaOH in 1000 mL of H_2O.
5 *N* H_2SO_4 solution: Dilute 140 mL of conc. H_2SO_4 to 1000 mL with H_2O.
1 *M* NaCl solution: Dissolve 58.5 g of NaCl in 1000 mL of H_2O (for batch of 24 make up 2 L).
Sodium dithionite ($Na_2S_2O_4$), analytical grade.
0.22 *M* sodium citrate/0.11 *M* sodium bicarbonate: Dissolve 67 g sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 9.3 g of sodium bicarbonate ($NaHCO_3$) in 1000 mL of distilled H_2O (for 24 samples make up 2 L).
1 *M* $FeCl_3$: Dissolve 70 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) in 1000 mL of dilute (about 0.2 *N*) hydrochloric acid.
Reagents for method of Watanabe and Olsen (1962) (Appendix A).
Reagents for method of Weaver (1974) (Appendix B).
Reagents for method of Harwood *et al.* (1969) (Appendix C).
Reagents for method of Fogg and Wilkinson (1958) (Appendix D).

(1) PROCEDURES FOR DETERMINING NONAPATITE INORGANIC PHOSPHORUS AND APATITE PHOSPHORUS IN SEDIMENTS

The following procedures are a modification of Chang and Jackson (1957).

(a) Procedure for Determining Nonapatite Inorganic Phosphorus

(i) Determination of Citrate-Dithionite-Bicarbonate (CDB) Extractable Inorganic Phosphorus

Weigh samples (0.5 g each) into 100-mL polypropylene centrifuge tubes.
Add 50 mL of 0.22 *M* sodium citrate/0.11 *M* sodium bicarbonate solution to each tube.
Immerse tubes in a water bath maintained at 85°C. After 15 min add 1.0 g sodium dithionite. Maintain the samples in water bath at 85°C for a further 15 min, stirring frequently with a glass rod.
Centrifuge the extract for 15 min at 2000 rpm (1100 *g*).
Transfer the supernatant solution quantitatively to 100-mL volumetric flasks, leaving the residue undisturbed in the tubes.
Add 25 mL of 1.0 *M* NaCl solution to each tube. Wash the residue well by mixing with Vortex mixer.
Centrifuge the extract at 2000 rpm for 10 min.
Transfer the extract to the volumetric flasks in the same manner as for CDB extracts (in some papers also referred to as DCB extracts). Save the residue for procedure (b) below.
Add 1 mL of 1 *M* $FeCl_3$ solution to each flask.
Permit flasks to stand exposed to atmosphere for two or three days (covered with filter paper or paper towels) until development of yellowish-brown colour indicates that the oxidation of the dithionite is complete.
Dilute the combined extract to 100 mL, shake well and determine orthophosphate in the extract by the method of Watanabe and Olsen (1962) or of Weaver (1974) (Appendices A and B).

(ii) Determination of NaOH Extractable Phosphorus

Add 50 mL of 1 *N* NaOH to the residue in the 100-mL centrifuge tubes.
Stopper, shake well and place tubes on an end-over-end or wrist action shaker overnight.
Centrifuge for 15 min at 2000 rpm (1100 *g*).
Transfer a 20-mL aliquot of the supernatant solution to another centrifuge tube.
Add 10 mL of 3 *N* HCl to the centrifuge tube containing 20-mL aliquot.

Mix with Vortex mixer and centrifuge for 10 min at 2000 rpm.
Transfer a 10-mL aliquot of the clarified supernatant to a 50-mL volumetric flask.
Determine phosphorus in solution by the method Harwood *et al.* (1969) (Appendix C).
Discard the rest of the NaOH extract.
Add 30 mL of 1 *M* NaCl to each tube containing the residue.
Wash the residue (using Vortex mixer).
Centrifuge the tubes for 10 min at 2000 rpm.
Discard the supernatant without disturbing the residue.

(b) Procedure for Determining Apatite Phosphorus (HCl-Extractable Phosphorus)

To the sample residue from procedure (ii) add 50 mL of 1 *N* HCl and shake overnight.
Centrifuge for 15 min at 2500 rpm (1700 *g*).
Transfer a 2-mL aliquot of the supernatant solution to a 50-mL volumetric flask.
Determine orthophosphate in the extract by the method of Harwood *et al.* (1969) (Appendix C).

(2) PROCEDURE FOR DETERMINING TOTAL AND ORGANIC PHOSPHORUS IN SEDIMENTS

These procedures are a modification of Mehta *et al.* (1954) and Sommers *et al.* (1972).

Extraction Procedure

Weigh 0.5 g sample into a 100-mL polypropylene centrifuge tube.
Add 50 mL of 1 *N* HCl and shake for 16 h (overnight).
Centrifuge at 2500 rpm for 15 min.
Transfer the supernatant solution to a 250-mL volumetric flask.
To the sample residue add 50 mL of 1 *N* NaOH and shake for 4 h.
Centrifuge at 2500 rpm for 15 min.
Transfer the supernatant solution to the same 250-mL flask.
To the sample residue add 50 mL of 1 *N* NaOH.
Place tube in an oven at 90°C for 16 h (overnight) covered to avoid evaporation.
Let cool and centrifuge at 2500 rpm for 15 min.
Combine the supernatant solution with the preceding HCl and NaOH extracts.
Add 6 mL of conc. HCl to the combined extracts, dilute to 250 mL and mix.

Determination of Orthophosphate in Extract

Transfer approx. 50 mL of combined extract to 100-mL centrifuge tube.
Centrifuge at 2500 rpm for 15 min.
Transfer 10-mL aliquot of the clarified solution to a 50-mL volumetric flask.
Determine orthophosphate by the method of Harwood *et al.* (1969) (Appendix C).

Determination of Total Phosphorus in Solution

Mix thoroughly the combined extract containing precipitated organic matter in the 250-mL flask.
Transfer a 10-mL aliquot of suspension to a 125-mL Erlenmeyer flask.
Evaporate the solution almost to dryness (approx. 1 mL residual solution).
Add 1 mL of 72% $HClO_4$.
Place on hot plate and digest.
When $HClO_4$ fuming commences (dense white fumes), place a small funnel stem downward in the mouth of the Erlenmeyer flask.
Continue $HClO_4$ digestion for 10 min or until no more particles of organic matter are visible.
Add 20 mL of distilled H_2O.
Digest solution at 90°C for 1 h.
Determine P in solution by the method of Fogg and Wilkinson (1958) (Appendix D).

Determination of Organic Phosphorus

The organic phosphorus of the sediment extracts is determined by the calculation of the difference between the total phosphorus in solution and the orthophosphate in extract.

RESULTS AND DISCUSSION

The procedure was tested by the analysis of four samples in the laboratory. Three of these were surficial lacustrine sediments and the other was a till sample from eroding bluff on the Lake Erie shore. Two of the sediment samples came from depositional basins in Lake Ontario (Table 1).

Table 1. Lake Ontario Sediment Sample Locations, Water Depths and Sedimentation Rates

Sample	Latitude	Longitude	Water depth (m)	Present day sedimentation rate (mm/yr)
WB	43° 24.10′ N	79° 43.64′ W	101	1.3
E-30	43° 30.7′ N	76° 54.0′ W	225	1.6

Table 2. Results of Laboratory Analyses (P content in milligrams per kilogram dry sediment)

Sample	(1) NAI-P CDB-P	(2) NAI-P NaOH-P	(3) Apatite-P HCl-P	(4)* (1)+(2)+(3)	(5)* Orthophosphate in combined extract	(6) Organic-P (by difference)	(7) Total-P
WB, Lake Ontario	547±22	50±7	365±20	962	987±14	303	1290±22
E-30, Lake Ontario	590±17	42±4	315±10	947	934±14	210	1144±19
Perch Lake	608±34	42±3	305±19	955	951±24	211	1162±14
Lake Erie till	25±9	7±4	328±5	360	375±11	2	377±20

* Note the good agreement between columns (4) and (5).

The other sediment sample is from a shield lake, Perch Lake. Perch Lake is a small, roughly heart-shaped lake on the property of Atomic Energy of Canada Limited (AECL) about five miles from the village of Chalk River. It is located on the part of the Canadian Shield known as Grenville Province. The lake is at the lower end of a small drainage basin (8 km^2), elongated northwest to southeast, and parallel to the Ottawa River into which it drains.

Table 2 contains the results obtained from analyses of these samples in the laboratory. Statistics were calculated from five to nine replicates.

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APPENDIX A

DETERMINATION OF ORTHOPHOSPHATE IN SOLUTION BY THE METHOD OF WATANABE AND OLSEN (1962)

PROCEDURE

Transfer aliquot of test solution (20 mg phosphorus as PO_4^{3-}) to a 125-mL separatory funnel.
Add 5 mL molybdate reagent.
Add H_2O to bring volume to 20 mL.
Add 20 mL 1-butanol.
Shake for 2 min and discard aqueous phase.
Add 10 mL of 1 *N* H_2SO_4.
Shake for 2 min and discard aqueous phase.
Add 15 mL of stannous chloride reagent.
Shake for 1 min and discard aqueous phase.
Transfer butanol phase to 25-mL volumetric flask.
Rinse separatory funnel with 10 mL of ethanol and transfer the rinse to the 25-mL flask.
Dilute solution in 25-mL flask to mark with ethanol and mix well.
Measure absorbance at 725 μm (10-mm absorption cells).

REAGENTS

Molybdate reagent: Dissolve 50 g solid $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL of H_2O. Add 400 mL of 10 *N* H_2SO_4 and dilute to 1 L.
1 *N* H_2SO_4: Dilute 100 mL of 10 *N* H_2SO_4 to 1000 mL with distilled H_2O.
Stannous chloride stock solution: Dissolve 10 g of $SnCl_2 \cdot 2H_2O$ in 25 mL conc. HCl. Store in a refrigerator.
Stannous chloride reagent: Dilute 1 mL of stannous chloride stock solution to 200 mL with 1 *N* H_2SO_4 immediately before use.
1-butanol $CH_3(CH_2)_2 \cdot CH_2OH$, analytical grade.
Ethanol, $CH_3 \cdot CH_2OH$, absolute analytical grade.
10 *N* H_2SO_4: Add 278 mL conc. H_2SO_4 to 754 mL H_2O.
Stock standard phosphorus solution: As for method of Harwood *et al.* (1969).
Dilute standard phosphorus solution: As for method of Harwood *et al.* (1969).
Take 2 mL aliquot for the "standard" funnel.

APPENDIX B

DETERMINATION OF ORTHOPHOSPHATE IN SOLUTION BY THE METHOD OF WEAVER (1974)

The procedure (Weaver, 1974) was modified by means of adding the mixed colorimetric reagent (see Harwood *et al.*, 1969). Add 5% ammonium molybdate solution before adding the mixed colorimetric reagent. The actual amount of ammonium molybdate solution depends on size of the CDB aliquot. For 50 mL of final solution add:

1 mL of 5% ammonium molybdate solution if CDB aliquot is 2 mL.
3 mL of 5% ammonium molybdate solution if CDB aliquot is 5 mL.

A 5% solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (mol. wt. = 1236.0) is 0.0405 *M*. The concentration of citrate in the CDB final extract is 0.114 *M*.

APPENDIX C

DETERMINATION OF ORTHOPHOSPHATE IN SOLUTION BY THE METHOD OF HARWOOD *ET AL.* (1969)

PROCEDURE

Place aliquot of solution containing 0–150 mg phosphorus as PO_4^{3-} (0–50 mg phosphorus as PO_4^{3-} is optimal) in a 50-mL volumetric flask.

Dilute to approx. 32 mL with H_2O.

Add 10 mL of colorimetric reagent.

Dilute to 50 mL and mix.

Measure colour intensity 30–120 min after colour development at 890 μm using 10-mm absorption cells.

REAGENTS

4 *N* H_2SO_4: Dilute 112 mL of conc. acid to 1 L.

Ammonium molybdate solution: Dissolve 32 g of $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ in 1 L of H_2O.

Ascorbic acid solution: Dissolve 5 g of solid in 50 mL H_2O. Make fresh solution each day.

Potassium antimonyl tartrate solution: Dissolve 2.7 g of solid in 250 mL H_2O. (Lifetime is limited by fungal growth.)

Colorimetric reagent (for 24 samples, standard, blanks): Combine 250 mL of 4 *N* H_2SO_4, 75 mL of ammonium molybdate solution, 25 mL of potassium antimonyl tartrate solution, 100 mL of 10% ascorbic acid solution, and dilute to 500 mL with distilled H_2O.

Stock standard phopshorus solution: Dry some KH_2PO_4 overnight at 40°C. Dissolve 0.4391 g in 1000 mL of 0.17 *N* HCl. 1 mL = 100 μg P.

Dilute standard phosphorus solution: Dilute 5.0 mL of stock standard solution to 50 mL in a volumetric flask with H_2O. Discard after three days. This standard contains 10 μg P/mL.

APPENDIX D

DETERMINATION OF ORTHOPHOSPHATE IN SOLUTION BY THE METHOD OF FOGG AND WILKINSON (1958)

PROCEDURE

Place aliquot of solution containing 0-50 mg phosphorus as PO_4^{3-} in a 125-mL Erlenmeyer flask.

Bring to approximate neutrality with additions of 5 *N* NaOH or 5 *N* H_2SO_4, using 2,4-dinitrophenol as indicator.

Dilute to approx. 34 mL with H_2O.

Add 10 mL ammonium molybdate reagent.

Add 1 mL ascorbic acid solution.

Heat solution to boiling point.

Cool and transfer to 50-mL volumetric flask.

Dilute to mark and mix well.

Measure absorbance at 820 μm using 10-mm (or if necessary 40-mm) absorption cells.

REAGENTS

Ammonium molybdate reagent: Dissolve 10 g of solid $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 100 mL H_2O. Add 300 mL of 1:1 H_2SO_4 and dilute to 1 L.

Ascorbic acid solution: Dissolve 5 g of solid in 50 mL H_2O. Prepare fresh daily.

2,4-dinitrophenol solution.

Stock standard phosphorus solution: As for method of Harwood *et al.* (1969).

Dilute standard phosphorus solution: As for method of Harwood *et al.* (1969).

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